DEVELOPMENTAL PATTERN AND ADAPTATIONS FOR REPRODUCTION IN *NUCELLA CRASSILABRUM* AND OTHER MURICACEAN GASTROPODS [1]

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An extensive literature about development in muricacean gastropods from the northern hemisphere and tropical coasts has been developed; however, many of these works are strongly descriptive and do not always present an adaptive interpretation of features observed. In recent years a valuable advance has been achieved by Spight, based on his extensive field experience with thaidids. Some muricid developmental rules proposed by Spight refer to factors influencing pre-hatching time, ecology of hatching size, hatching type in relation to latitude and habitat conditions as well as factors conditioning selection of spawning sites (Spight, 1975, 1976b, 1977a, c).

To determine how far these rules are applicable in controlling the evolution of developmental patterns in muricaceans requires more extensive comparative knowledge of species from geographical areas not yet explored. In this sense, the coast line of Chile is very promising, as it comprises an extensive latitudinal range accompanied by great diversity in habitat conditions. Some years ago, a comparative study of reproduction in Chilean muricids was begun. Preliminary interest was focused on the commercially important species *Concholepas concholepas* (Gallardo, 1973; Gallardo, in press). Later, egg masses, embryo feeding and hatching type of *Chorus giganteus* were also analyzed; these results have been discussed in relation to habitat conditions (Gallardo, in press). In the present paper, studies on egg masses and embryos of the intertidal snail *Nucella crassilabrum* from the locality of Mehuín, a small bay near Valdivia, are reported; this information is complemented with field observations on habitat, spawning sites, embryo mortality and pre-hatching time at two different seasons. A discussion follows in order to interpret some of these features; hatching type and hatching size are analyzed in relation to the rules earlier set out by Spight. Emphasis is given to embryo feeding patterns and their possible adaptive significance within the holobenthic muricaceans, a question still not completely answered.

MATERIALS AND METHODS

Egg capsules of *Nucella crassilabrum* were collected on the intertidal rocky shore of Mehuín (39° 25′ S, 73° 10′ W) from March, 1976 until April, 1977. Capsules of one cluster were separated and monitored to record capsule size composition as well as synchronism of development between different capsules in each group. Development of embryos was followed by opening capsules in different
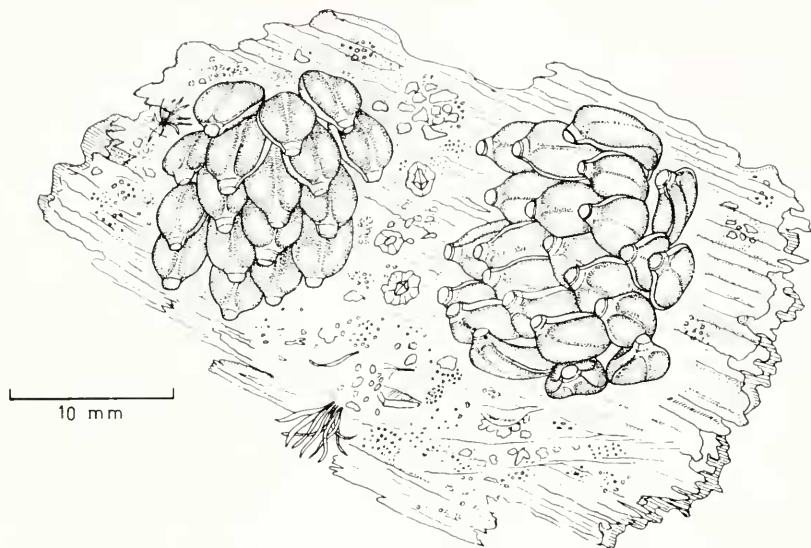
FIGURE 1. *N. crassilabrum.* Egg masses attached on vertical rocky substrate at the intertidal of Mehuín.

embryonic phases. Measurements and drawings were made from living material observed under a stereo-microscope provided with a micrometer eyepiece. Number of nurse eggs ingested per embryo was estimated from capsules in which all nurse eggs had been eaten; careful dissections of embryos in early trochophore phase made it possible to determine the number ingested and its variation.

Pre-hatching time was recorded in clusters that were spawned at different seasons of the year. For this purpose, two egg masses containing eggs in early cleavage stage were selected and tagged. One of these was spawned during late autumn and the other during late spring. Each spawning site was periodically visited on tidal exposure periods until the snails had hatched; on each visit, 3 or 4 capsules were collected for further examination at the laboratory. Water temperature of the sea is recorded daily at the Laboratory of Mehuín; this is the information used for the present paper. Spawning sites used by *N. crassilabrum* were inspected to record habitat preferences. Embryos killed by physical stresses were identified by their pink color.

RESULTS

Egg capsules and masses

Shape of the egg capsule of *Nucella crassilabrum* resembles that of its congener, *N. lapillus* (Ankel, 1937). Each flattened capsule has concave and convex sides; when seen from either the convex or concave side, the structure above the peduncle appears nearly oval in shape, with a gradual increase in breadth toward the top. At the top of the capsule there is a circular exit hole which is closed by a

prominent plug. It is possible that, as observed in other neogastropods (Hyman, 1967), this plug weakens and dissolves as the embryos reach the hatching stage. The exit hole diameter is approximately 875 to 1200 μm. Inside the capsule, eggs and mucus-like fluid are contained in a thin transparent sac. Each capsule possesses a short stalk; stalks of various capsules are cemented to the substrate in a continuous band. The capsule wall is fairly transparent, showing the embryos inside. Clusters are yellowish, due to the yellowish eggs when freshly laid, and fade to dull grey with the development of the larvae. Size of egg capsules depends on size of the female producing them. The length of the capsules we have observed, excluding the stalk, varies between 5.0 to 12.8 mm.

The capsules are laid very close to each other with a distance of approximately 2.0 to 2.5 mm between stalks. Field observations suggest that, as typical in certain muricids, communal spawning is also the rule in this species. The clusters (Fig. 1) are laid close to each other, making it difficult to ascertain the number of egg capsules laid by a single female. Nevertheless, in certain cases, the orientation and capsule size allow one to distinguish egg masses laid by different females; such clusters may contain up to 60 egg capsules. Capsules of the same cluster are arranged in a definite pattern, all of them facing the same direction; capsules arranged in a given row alternate in position with respect to those from a contiguous row as seen in Figure 1. Direction in which the different clusters are oriented on the substrate appears random.

Spawning sites

Egg masses are attached to rocky substrates, most frequently in crevices and on vertical surfaces and least frequently in tidepools and on horizontal surfaces. In general, permanently wet and shaded intertidal sites, from extreme low water of spring tides to about mean low water of neap tides, are preferred. Capsules at this last intertidal level were found in a group of rocks partially buried in the soft sandy beach. In this area, extreme seasonal sand fluctuations of about 80 or 100 cm are observed and a great quantity of sand is deposited during the summer, greatly reducing the rocky surface merging above the soft bottom. In that case, many egg masses of *Nucella crassilabrum* are covered by the sand. Furthermore, the retreating tide regularly exposes them to dry air and wind, especially throughout late spring and summer; snails become exposed to air longer and more frequently than lower on the shore and many egg masses do not complete development, probably owing to the effect of these environmental stresses. Capsules with embryos killed by physical stresses change from the normal yellow color to pink or purple. The most favorable situation for subsequent hatching of capsules was at those sites where they were permanently submerged in the sea water.

Eggs and embryos

The eggs are creamy white, their diameter varying between 204 and 293 μm with a mean of 240 μm. The number of eggs per capsule varies from 134 to 1116. A significant correlation observed between capsule length and number of eggs per capsule (Fig. 2) accounts for this variation. Besides the normally viable eggs there are many others that undergo atypical development and serve as nurse eggs.
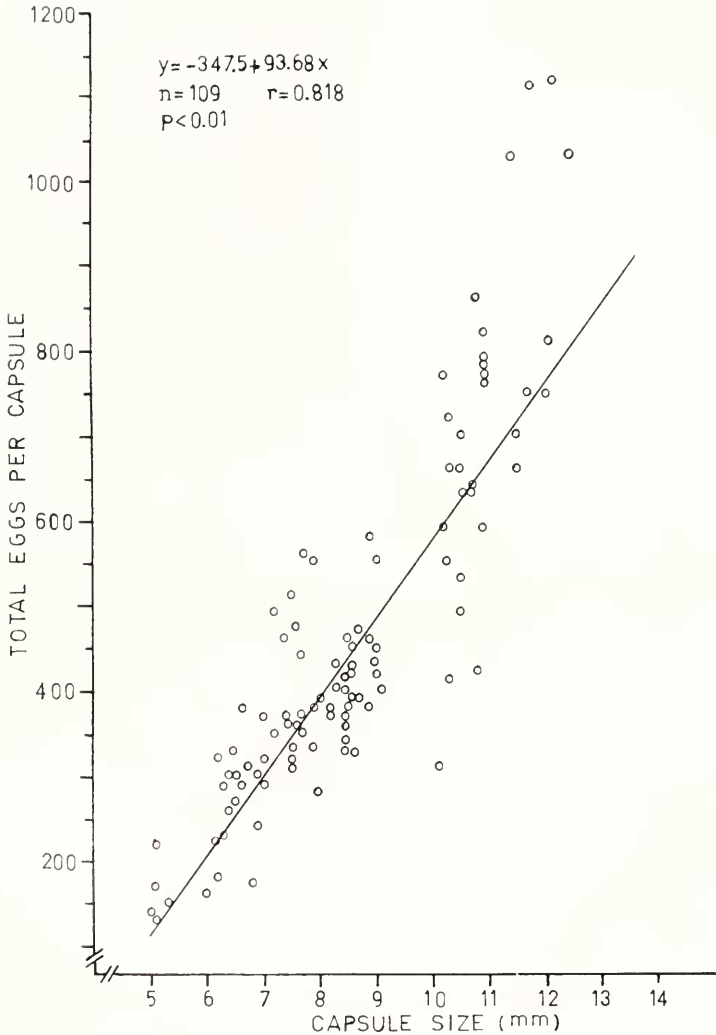
FIGURE 2. *N. crassilabrum.* Relationship between number of eggs per capsule and capsule size.

Counts in three egg capsules of different sizes (Table I) reveal that only 6.6 to 7.9% of the total eggs in a capsule are viable. The number of embryos also increases in proportion to capsule size (Fig. 3); it varies between 10 and 122.

The intracapsular development of *N. crassilabrum* is of direct type. In viable embryos it follows the normal spiral pattern of cleavage. On the other hand, atypical cleavage is observed in non-viable nurse eggs which appear very variable in shape. The atypical development of these nurse eggs is finally arrested. The fertile larvae begin ingesting nurse eggs when they attain an early trochophore stage. By then, the mouth, esophagus and body expand to enclose entire nurse eggs as they are pushed down the digestive tract by the cilia lining it. The

TABLE I.

Nucella crassilabrum. *Percentage of viable embryos and nurse-eggs in three different capsules.*

Capsule size (mm)	Total nurse eggs	Viable embryos and percentage
7.9	406	32 (7.3%)
6.8	342	24 (6.6%)
8.6	397	34 (7.9%)

ingested eggs are visible as distinct bulges in the body wall (Fig. 4b) and will spill out intact from an embryo opened during the feeding period. In the succeeding stages (Figs. 4c, d) and intracapsular veliger is gradually developed; by then, the embryos show the appearance of the shell and foot and the velar lobes are partially expanded. The embryo is now clearly divided into head (anterior to the shell), foot (ventrally), and visceral hump (covered by the shell); nurse eggs ingested still obscure details of internal structure (Fig. 4e). The posterior
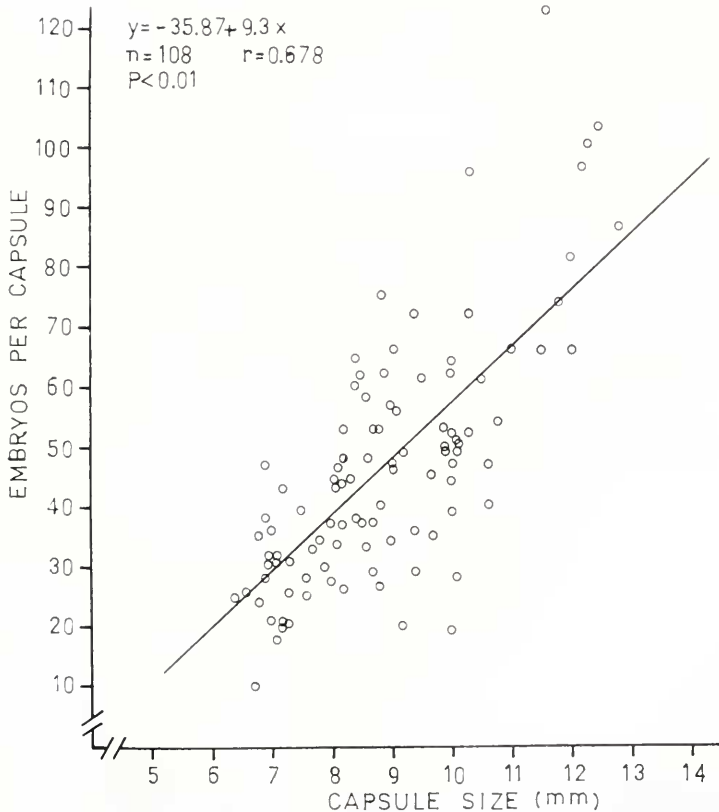

FIGURE 3. *N. crassilabrum.* Relationship between number of viable embryos per capsule and capsule size.
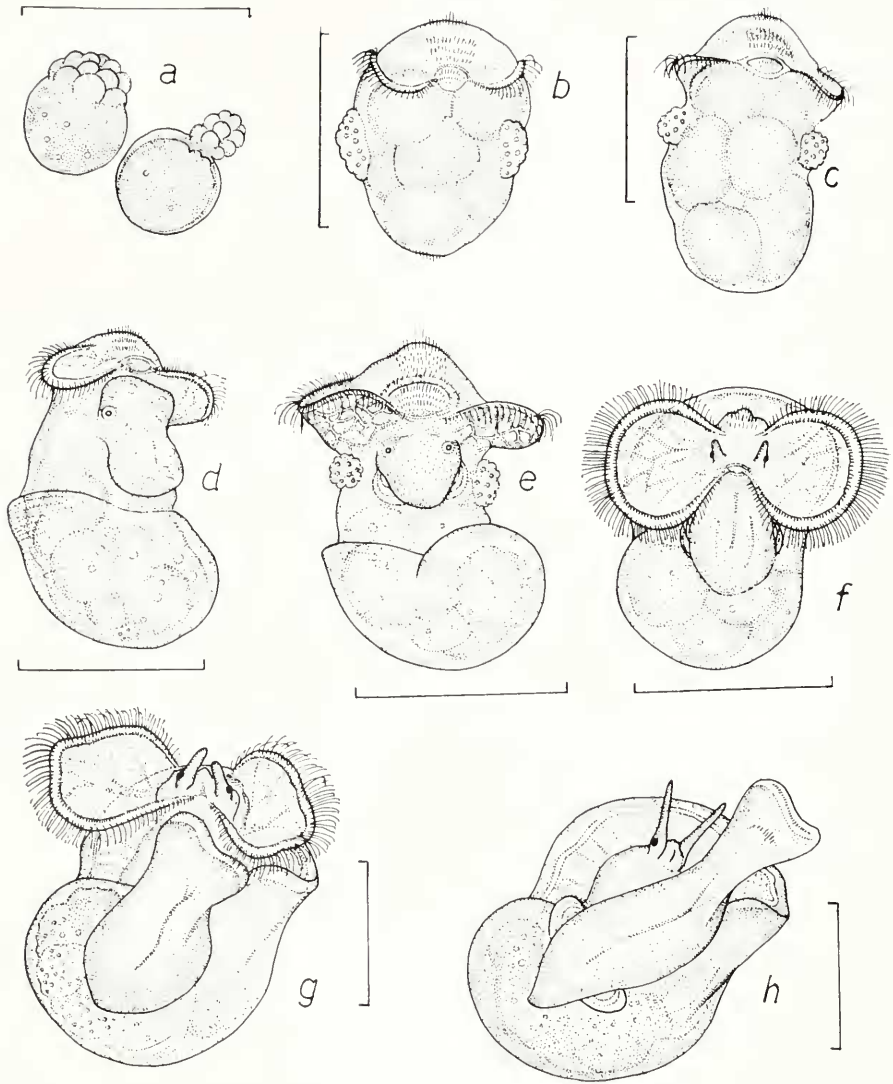
FIGURE 4. *N. crassilabrum.* Different stages of intracapsular development. a) a fertile egg (left side) next to a nurse-egg, in cleavage stage. b) trochophore stage with the outlines of whole nurse-eggs seen through the body wall. c–d) early veliger stage. e–f) mean and advanced veliger stage. g) pre-hatching stage with the velum in reabsorption process. h) hatching juvenile stage. The lines equal 500 µm.

face of the foot gradually differentiates a small, thin operculum. On either side of the foot a spherical statocyst is visible. Within the velar rim and around the stomodaeum, rudiments of the adult head are now beginning to become organized and a pair of black eyespots is visible on the base of recently developed tentacles

(Fig. 4f). Ingestion and feeding activity may continue in some capsules up to the veliger stage illustrated in Figure 4e.

The next stages of the intracapsular veliger (Figs. 4g, h) show a gradual and extensive increase in size, especially of the shell, foot and tentacles. The yolk gradually disappears in the visceral hump with accompanying differentiation of the viscera. A columella muscle faintly visible on the left side of the visceral hump is able to effect withdrawal of both head and foot into the shell. By the end of intra-capsular development, the lateral lobes of the velum have been resorbed and this pre-hatching juvenile shows an active crawling foot and large tentacles.

Embryonic feeding and rate of development

During intracapsular development, some embryos acquire more nurse eggs than their capsulemates and this is reflected in the size distribution of embryos and hatchlings. Embryos in trochophore phase as small as 450 μm were found together with others as large as 775 μm after all nurse eggs had been eaten. By this time, the number of eggs eaten by each larva varies from 3 to 20 as shown in Figure 5 (left side) for three capsules with different numbers of capsulemates. This figure also shows the frequency at which embryos ingest different numbers
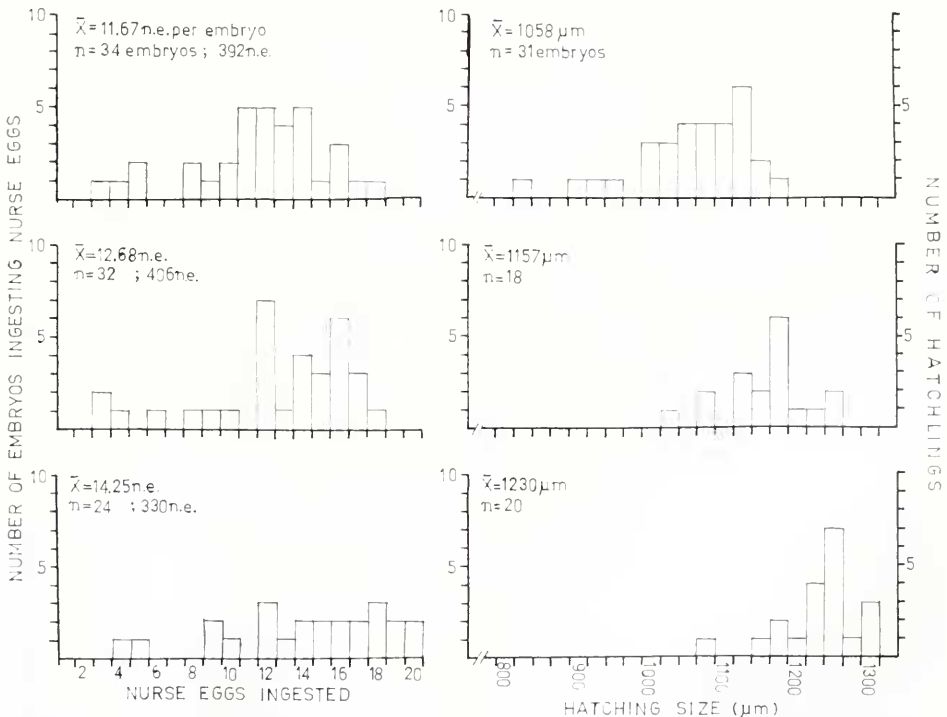

FIGURE 5. *N. crassilabrum.* Left, frequencies of embryos ingesting different numbers of nurse-eggs in three different capsules. Right, hatching size frequencies between embryos of three different capsules. n.e. = nurse-eggs.

of nurse eggs. Hatching size distributions for juveniles from three different cap-
sules are shown in Figure 5 (right side). While some juveniles attain 825-μm
shell length at the moment of hatching, others are as large as 1300 μm. Mean
number of nurse eggs ingested per embryo per capsule as well as mean hatching
size, seem to change regularly as the number of embryos per capsule decreases.
The effect of haphazard distribution of embryos among capsules on hatching size,
has been previously studied by Spight (1976a) in the muricids *Thais emarginata*
and *Acanthina spirata*. While embryo counts are less predictable in these species,
nurse eggs per capsule are distributed more regularly. Some embryos share their
yolk supplies with many more capsulemates than others and this factor is most
important in determining a variable hatching size between nurse egg feeders'
embryos (Spight, 1976a).

Time required to complete intracapsular development was estimated from two
egg masses spawned at different seasons of the year. At the time the observations
began, the egg capsules were in an early cleavage stage. An egg mass controlled
during autumn and winter months took between 70 and 80 days to fully develop
and hatch; mean of monthly water temperature from May to August, 1976 at
Mehuín, varied between 9.67 and 10.60° C. In other masses that developed during
late spring and early summer, the time was significantly reduced to approximately
55 to 65 days; in this case, mean of monthly water temperature from November,
1976 to January, 1977 varied between 11.40° and 14.10° C. Doubtlessly, dif-
ferences in developmental rates in nature are well accounted for by seasonal changes
in sea water temperatures at Mehuín.

Discussion

Nucella crassilabrum shows the most evolved pattern of development known for
Chilean muricids if we compare it with observations in *C. concholepas* (Gallardo,
1973) and in *Ch. giganteus* (Gallardo, in press). The type of development of
N. crassilabrum agrees with that reported for its congener *N. lapillus* (Pelseneer,
1910). Such pattern of development is expected in high latitude muricaceans
inhabiting interidal rocky shores (Spight, 1977a). Spight establishes that, among
the rocky intertidal muricaceans, the hatching type evolves markedly according to
a latitudinal gradient; while in this habitat all tropical species maintain planktonic
larvae, all high latitude species metamorphose before hatching. In this sense,
N. crassilabrum, whose geographic distribution extends along the Chilean coast
to the Magellan Strait, may be included within the muricid group showing such
reproductive tendency. In a paper about hatching type of *Chorus giganteus*
(Gallardo, in press), a case of coincidence with Spight's predictions for muricaceans
from another type of habitat has also been reported. According to Spight (1977a),
muricaceans with an intermediate hatching type (a non-feeding veliger near to
metamorphosis) had been found to inhabit a shallow water sand habitat; the
findings in *Ch. giganteus* agree, as this sand bottom muricid also possesses such
hatching type. Causes for these tendencies in muricid developmental patterns when
related to latitude and habitat conditions are unknown.

All reproductive patterns should involve adaptations to maximize survival of
embryos and, consequently, their reproductive fitness. Some of these adaptations

should include ability of adults to discriminate and choose good spawning sites, as well as an appropriate developmental time and hatching size. Our field observations in *N. crassilabrum* reveal that mortality of embryos is of common occurrence at least at the upper shore spawning sites. Probably some sites offer more protection than others; for instance, one would expect physical stresses to be minimal in tidepools, but they are used less frequently than vertical surfaces exposed to repeated dehydration during low tides. According to observations of the intertidal muricid *Thais lamellosa* (Spight, 1977c), spawning sites selected would reflect the conflicting demands of different life history stages. According to Spight, vertical surfaces are least accessible to predators and often are covered with food (barnacles), but provide little protection from physical stresses. Crevices offer some protection from physical stresses and usually are covered with barnacles but are accessible to predators. Understone surfaces and tidepools offer protection from stresses but are accessible to predators and are often distant from food sources. With these considerations in mind, Spight concludes that *T. lamellosa* females deposit capsules most frequently on the surfaces that are most suitable when the demands of all life history stages are considered. Studying the congeneric species *N. lapillus*, Feare (1970) found that physical stresses operating during exposure to air are responsible for hatching success of 0% at mean tide level and 57% at mean low water of neap tides. Our field observations in *N. crassilabrum* suggest that this species also uses poor spawning sites regularly and it probably does so for the same reasons that *T. lamellosa* does. For instance, different environmental stresses seem to be influencing survival of *N. crassilabrum* adults when closely located sites are compared (Gallardo, in preparation); demands of this life history stage could be in part conditioning the selection of spawning sites that are most suitable.

Hatching size of intertidal muricaceans is shorter (0.6 to 1.3 mm; $\bar{X} = 1.01$ mm) than that of species living in subtidal habitats (0.6 to 2.5 mm; $\bar{X} = 1.54$ mm) or on coral reef flats (1.1 to 1.9 mm; $\bar{X} = 1.40$ mm) [Spight, 1976b; $(\bar{X}) =$ mean values calculated from a few species where data are available]. According to this, the hatching size of *N. crassilabrum* (0.82 to 1.30 mm) corresponds with the habitat this muricid occupies. To attain the appropriate hatching size, holobenthic muricaceans have followed two evolutionary paths: provision of nurse eggs or increase in size of a self-sufficient fertile egg. Now the question arises: which selective forces favor one or the other of these developmental options? It has been hypothesized that providing much of the yolk as nurse eggs may allow a snail to have a large hatching size and at the same time a relatively brief developmental time (Spight, 1975). In order to prove the validity of this hypothesis between muricaceans, we may use information available about developmental time, hatching size and embryonic feeding source both in *N. crassilabrum* (this paper) as in other species (Spight, 1975, 1976b); this information is compared in relation to habitat. We may see that among intertidal muricaceans adaptation for nurse egg feeding is of common occurrence; in a few species of this habitat, energy for developing embryos is totally obtained from larger fertile eggs (*Thais lamellosa* 590 μm; *T. canaliculata*, 620 μm). *T. lamellosa* reveals a developmental time of 67 to 91 days at 9.6° to 11° C, more or less similar to that of *N. crassilabrum*, although the upper limit of its range, 91 days, slightly exceeds that of *N. cras-*

silabrum; 80 days at 9.6° to 10.6° C. On the other hand, subtidal species usually require a large hatching size. To attain it, they usually have evolved a large fertile egg (*Ceratostoma foliatum,* 720 μm; *Torvamurex territus,* 675 μm); numerous nurse eggs per embryo are consumed in species of this habitat showing such feeding mechanism (*Murex senegalensis,* 35 n.e. per embryo; *M. quadrifrons,* 91 n.e. per embryo). Subtidal species whose egg exceeds 600 μm in diameter, reveal a markedly slow developmental rate; for instance, *T. territus* takes 90 days to hatch at 20° C and *C. foliatum* takes 120 days to hatch at 10° to 12° C. Our observations in *N. crassilabrum* and those of Spight (1977c) and Feare (1970) reveal that one of the most important sources of embryo mortality in muricaceans laying in the intertidal, are the physical stresses operating at this level; an embryo of *T. lamellosa* cannot tolerate even one two-hour exposure (Spight, 1977a). Similar observations have been made by Pechenik (1978) in prosobranchs of the family Nassidae. Pechenik found that egg capsules of *Nassarius obsoletus* did not afford substantial protection against desiccation; 0.5-hr exposures to 75% relative humidity killed 17.5% of the embryos. It is reasonable to assume that such mortality would be reduced by laying the egg capsules in protected micro-environments, as *N. obsoletus* clearly does (Pechenik, 1978), or by shortening developmental time of clusters spawned at more exposed sites of the intertidal. When, for reasons considered above, an intertidal muricid does not always use good spawning sites for embryos, the possibility that it is shortening developmental time should be preferred. An evolutionary option in this sense may be to change to another form of embryonic feeding, that is the provision of nurse-eggs. But, how to explain nurse-egg feeding by some subtidal muricaceans? In this case, this embryonic feeding adaptation seems to have been specially favored to attain an extremely large hatching size. Relatively large muricid eggs range from 675 to 920 μm in diameter (*T. territus, Thais lima*); nevertheless the hatchlings developed from such eggs do not exceed from 1.3 to 1.5 mm in length. In subtidal species, usually requiring a larger hatchling (1.6 to 2.0 mm), each embryo is provided with numerous nurse eggs (*M. quadrifrons, M. senegalensis*). These preliminary considerations suggest that a delicate compromise between at least the optimal hatching size and developmental time, could be orienting evolution of embryonic feeding adaptations among holobenthic muricaceans. However, further work is necessary to reinforce this hypothesis. The results for the small number of species considered in this discussion cannot, by themselves, support it, but they can add to the data for future comparative studies. Information about developmental time, hatching size, habitat, and mortality sources is still lacking in various muricids. An optimal material to evaluate the advantages of evolving nurse eggs is offered by *T. canaliculata*; this intertidal snail appears to be in the course of evolving from one reproductive mode (self-sufficient large egg) to the provision of nurse-eggs for embryos (Spight, 1977b).

SUMMARY

1. Eggs of *Nucella crassilabrum* range from 204 to 293 μm in diameter (mean = 240 μm). Only 6.6 to 7.9% are fertile; the remaining are ingested as nurse eggs.

2. Embryos metamorphose before hatching. Pre-hatching time ranges from 55 to 80 days according to seasonal temperature fluctuations.

3. Hatching size varies from 0.82 to 1.3 mm, depending on number of nurse-eggs ingested per embryo (from 3 to 20). Number of fertile embryos per capsule (10 to 122) depends on capsule size.

4. Hatching type and hatching size shown by *N. crassilabrum* agree with those of other muricaceans living in similar habitat conditions.

5. Pre-hatching time and hatching size data of various muricaceans are analyzed to determine to what extent they influence embryonic mode of nutrition, namely the presence of nurse-eggs or alternatively large and fertile self-sufficient eggs. Provision of nurse-eggs for embryos is of common occurrence among intertidal muricaceans and this mode of nutrition seems to have been favored in such habitats to reduce developmental time. Providing the yolk as nurse-eggs seems also to contribute to a larger hatching size, as suggested by some subtidal muricaceans with such embryo support patterns.

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